



# Talking SMAAC: A New Tool to Measure Soil Respiration and Microbial Activity

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Soil respiration measurements are widely used to quantify carbon fluxes and ascertain soil biological properties related to soil microbial ecology and soil health, yet current methods to measure soil respiration either require expensive equipment or use discrete spot measurements that may have limited accuracy, and neglect underlying response dynamics. To overcome these drawbacks, we developed an inexpensive setup for measuring CO<sub>2</sub> called the soil microbial activity assessment contraption (SMAAC). We then compared the SMAAC with a commercial infrared gas analyzer (IRGA) unit by analyzing a soil that had been subjected to two different management practices: grass buffer vs. row crop cultivation with tillage. These comparisons were done using three configurations that detected (1) *in situ* soil respiration, (2) CO<sub>2</sub> burst tests, and (3) substrate induced respiration (SIR), a measure of active microbial biomass. The SMAAC provided consistent readings with the commercial IRGA unit for all three configurations tested, showing that the SMAAC can perform well as an inexpensive yet accurate tool for measuring soil respiration and microbial activity.

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## INTRODUCTION

Increased soil respiration due to warmer temperatures may exacerbate global climate change (Rustad et al., 2000; Davidson and Janssens, 2006; Bond-Lamberty et al., 2018), as soils currently have an gross efflux of  $\sim 60$  Gt C yr<sup>-1</sup> and represent one of the two largest terrestrial sources of carbon fluxes. Sequestering more carbon in soils has become a goal of climate mitigation efforts, such as the *four per mille* initiative (Minasny et al., 2017), and with particular emphasis on soils that have been degraded by human activities (Lal, 2004). Soil respiration measurements can help to inform such sequestration efforts, while also providing a means to monitor the health, and function of agricultural soils (Mondini et al., 2010; Allen et al., 2011). In the laboratory, soil respiration measurements are used to interpret soil microbial characteristics, for example using assays like SIR (Bradford et al., 2010), carbon mineralization (Song et al., 2014), and catabolic response profile (Casas et al., 2011).

Soil respiration is often assessed by measuring changes in carbon dioxide (CO<sub>2</sub>) concentration within a controlled volume over some period of time, and rely on either spot samples or integrated measurements. Spot samples are often analyzed using gas chromatography (GC) techniques (McGowen et al., 2018). Multiple GC measurements can also be combined for integrated measurements. However, these GC measurements can be costly, particularly

**Abbreviations:** IRGA, infrared gas analyzer; SIR, substrate induced respiration; SMAAC, soil microbial activity and assessment contraption.

when many samples are required. IRGA devices provide integrated flux measurements, and have been widely used to quantify soil respiration in forest (Gaudinski et al., 2000; Ladegaard-Pedersen et al., 2005; Don et al., 2009) and agricultural ecosystems (Smukler et al., 2012). IRGA-based measurements have also been used to study microbial community composition (Fierer et al., 2003), which represents one of the important properties related to soil function (Mukhopadhyay et al., 2014). While IRGA-based devices provide the most accurate flux data (Rowell, 1995), such sensors are often expensive, putting them beyond the means of many practitioners, and power-intensive, limiting their usefulness in the field.

Integrated measurements can also be collected using chemical titration with potassium hydroxide, KOH, or sodium hydroxide, NaOH (Haney R.L. et al., 2008). While titration methods are straightforward and can be done without expensive devices, there are concerns over the accuracy of the titration process (Haney R. et al., 2008). These methods often under-estimate soil respiration when compared to IRGA measurements (Ferreira et al., 2018). To add to this, titration methods often require substantial labor and laboratory space to conduct.

Finally, both spot and integrated samples can be analyzed using colorimetric techniques. For spot samples, colorimetric tubes can be used (Patil et al., 2010), while colorimetric paddles can provide integrated flux measurements (Sciarappa et al., 2016; Norris et al., 2018). Micro-respiration measurements, which quantify soil respiration and microbial community physiological profiles using indicator dyes in agar gel, also use colorimetric techniques (Campbell et al., 2003; Renault et al., 2013). Even though individual sampling units are relatively inexpensive, the materials are not re-usable and quickly become cost-prohibitive as the numbers of samples rise.

To address the above-mentioned shortcomings, we present an inexpensive Arduino-powered and IRGA-based CO<sub>2</sub> measurement device, called the soil microbial activity assessment contraption (SMAAC). The SMAAC has considerable flexibility, as we demonstrate using three different configurations: (1) SMAAC-Field, where the device was used to quantify soil respiration in a field setting; (2) SMAAC-Burst, where the device was used to analyze CO<sub>2</sub> evolution upon rapid re-wetting of air-dried soil; and (3) SMAAC-Biomass, where the device was used to quantify SIR. To validate these configurations, we compared the measurements provided by the SMAAC with those from a commercial field-portable IRGA system. These examples reveal that the SMAAC can perform well as an inexpensive yet accurate tool to measure soil respiration.

## MATERIALS AND METHODS

### **Soil Microbial Activity Assessment Contraption (SMAAC) Description and Calibration**

The sensor platform consists of four main components (**Figure 1**).

- (1) Arduino Uno (Arduino LLC, Ivrea, Italy).

- (2) Adafruit Data Logger Shield (Adafruit Industries, New York, NY, United States).
- (3) Sandbox Electronics 10,000 ppm CO<sub>2</sub> sensor (Sandbox Electronics, China).
- (4) 5 V DC power source.

The Arduino Uno is an open source/open hardware microcontroller based on the ATMEGA 328P. It has no storage space or accurate time-keeping abilities on its own, so the data logger shield contains a real time clock (RTC) and additional circuitry to store data on a removable SD card. The SMAAC was powered using four 1.5 V AA batteries. This configuration provided up to 21 h of readings at the rate of 20 readings per minute.

The CO<sub>2</sub> sensor requires only 4 wires to communicate with the Arduino (+V, RX, TX, and Ground). The sensor uses I2C (Intra Integrated Circuit) serial protocol and determines CO<sub>2</sub> concentration using non-dispersive infrared absorbance (NDIR). Example code for integrating this sensor with the Arduino is available at <https://github.com/SandboxElectronics/NDIRZ16>.

The CO<sub>2</sub> sensor has an option to calibrate itself to 400 ppm CO<sub>2</sub> based on ambient readings. To verify that this first-order calibration is accurate enough for scientific use, we checked the sensor accuracy using known CO<sub>2</sub> standards ( $n = 2$ ). Here, the sensor was installed via a rubber stopper into a 1 L jar (**Figure 2a**). The jar was filled with CO<sub>2</sub>-free air, and then 0.1 L of 1000 ppm CO<sub>2</sub> gas was replaced within the jar (providing a 100 ppm concentration within the jar). This process was repeated a second time with 1000 ppm CO<sub>2</sub> air, and also two times each with 2000 and 5000 ppm CO<sub>2</sub> air (providing concentrations of 200 and 500 ppm within the jar). The results obtained from SMAAC for these standards were repeatable within  $\pm 20$  ppm and accurate within the  $\pm 50$  ppm sensor limit.

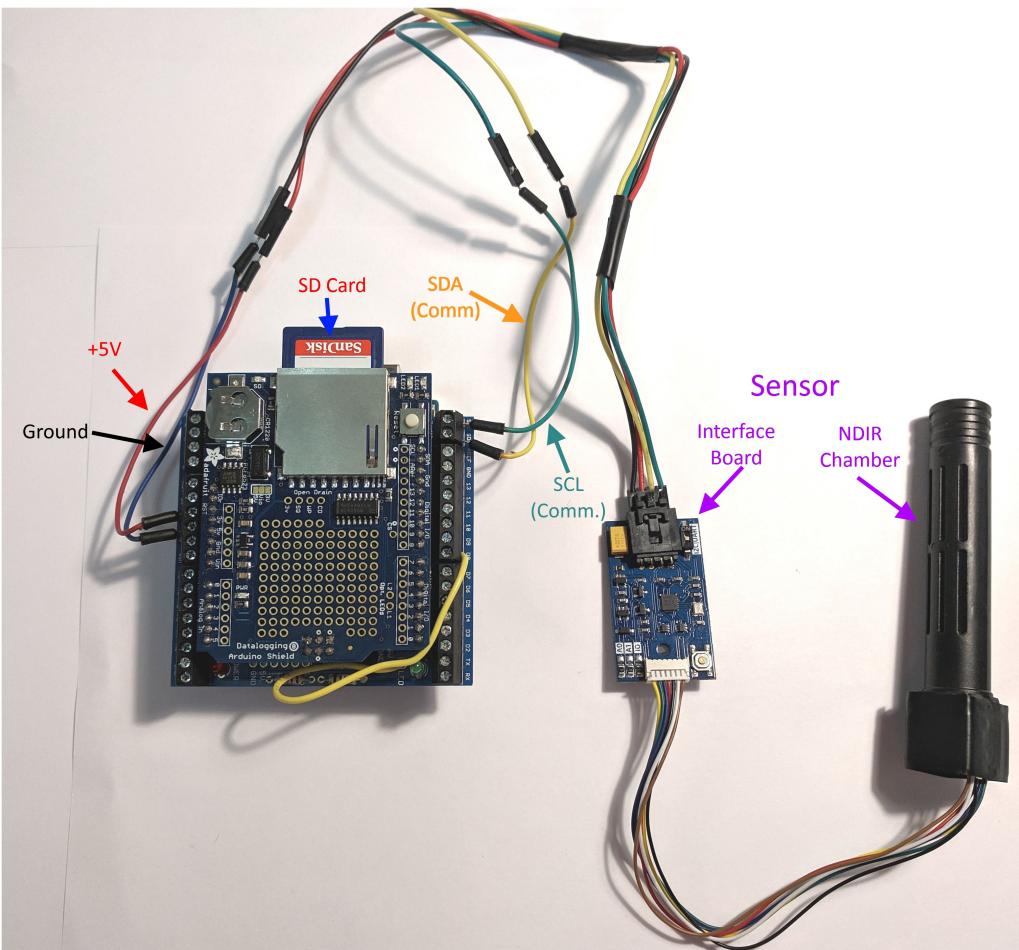
### **Soil Description**

We tested the SMAAC with a Weaver series silt loam soil (*Fine-loamy, mixed, active, and mesic Fluvaquentic Eutrudepts*), located at Kentland Farm at Virginia Tech (37.198, -80.575). To include different soil microbial activity levels, we sampled two locations in adjacent fields that were managed using (1) perennial grass cover and (2) row crop cultivation with moldboard tillage. The pH of the grass-covered soil was 6.4 and of the tilled soil was 6.6, putting the soil at the upper pH limit for performing static chamber measurements [e.g., West and Sparling (1986) recommend pH  $\leq 6.5$ ]. We performed three tests in which the SMAAC measurements were compared to a commercially available self-contained IRGA unit (LI-COR 8100 with 20 cm diameter 8100-8103 survey chamber, LI-COR, Lincoln, NE, United States): SMAAC-Field, SMAAC-Burst, and SMAAC-Biomass.

### **Field and Laboratory Measurements**

#### **SMAAC-Field Soil Respiration Test**

We used 200 mm (diameter) by 150 mm (height) PVC columns for the field measurements. We collected a 2-min CO<sub>2</sub> respiration measurement first using the SMAAC located within the LI-COR 8100-8103 sampling chamber (i.e., SMAAC-simultaneous;



**FIGURE 1 |** Schematic of the soil microbial activity assessment contraption (SMAAC). NDIR, non-dispersive infrared sensor used to detect CO<sub>2</sub>; SCL, serial clock line, used to synchronize data and commands between the Arduino and the interface board; SDA, serial data line, used to send and receive serial data and commands to the interface board.

**Figure 2b).** Note that the sampling chamber provided an air-tight seal around the PVC column during measurements. Immediately after this first measurement the LI-COR unit was removed and the ring was capped with an airtight rubber cap (i.e., SMAAC-independent; **Figure 2c**). The SMAAC then collected a second 2-min measurement. The CO<sub>2</sub> flux [ $f_{\text{CO}_2}$ ; (N L<sup>-2</sup> t<sup>-1</sup>)] was estimated as:

$$f_{\text{CO}_2} = \frac{P_0 V_c}{RT_0 A} \frac{\Delta C}{\Delta t} \quad (1)$$

where  $P_0$  is the pressure in the chamber [M L<sup>-1</sup> t<sup>-2</sup>], assumed to be equal to atmospheric pressure,  $V_c$  is the volume of the sampling chamber plus any tubing and pumps [L<sup>3</sup>],  $R$  is the ideal gas law constant [M L<sup>2</sup> N<sup>-1</sup> T<sup>-1</sup> t<sup>-2</sup>],  $T_0$  is the temperature of the air [T],  $A$  is the area of exposed soil [L<sup>2</sup>], and  $\Delta C$  is the change in CO<sub>2</sub> concentration on a molar basis [N N<sup>-1</sup>] per change in time  $\Delta t$  [t].

Four rings were sampled for each of the grass-covered and tilled soils ( $n = 4$ ).

### SMAAC-Burst CO<sub>2</sub> Test

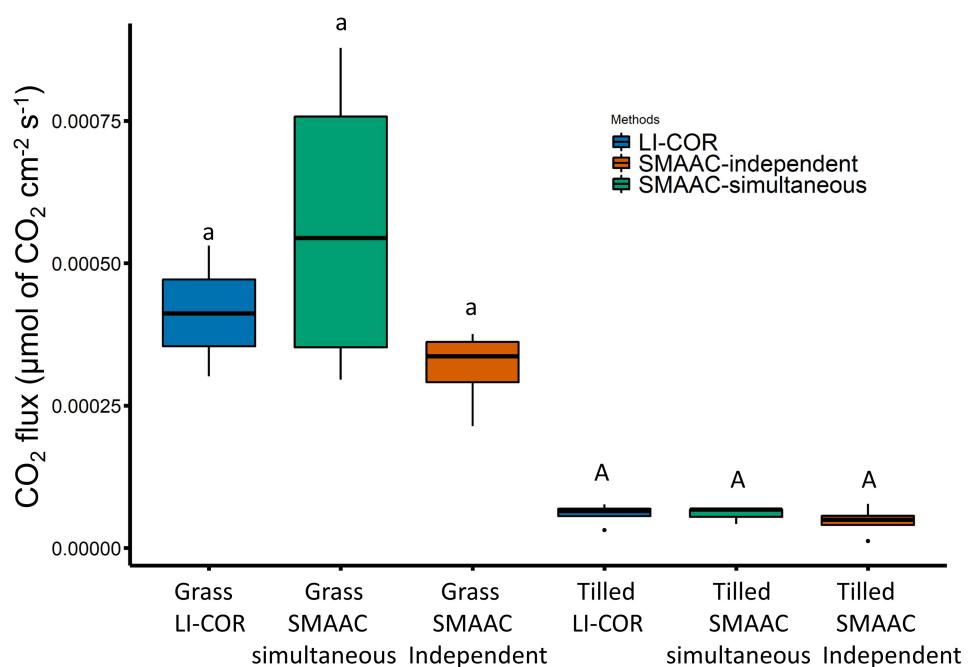
For the CO<sub>2</sub> burst test, we placed 200 g of 4-mm sieved and air-dried soil from the two sites into a 200 mm diameter by 150 mm tall column. The water holding capacity for each soil sample was measured using the funnel method (Fierer et al., 2006). Water was added dropwise to each soil sample using a syringe until the sample reached 50% water holding capacity. Once the soil samples were wetted, the SMAAC was placed on the soil surface (**Figure 2d**). The LI-COR 8100 sampling hood was then placed on top. Both instruments collected readings several times a minute for at least 2 h. For each instrument, the readings collected were averaged per minute for graphing purposes ( $n = 4$  per soil).

### SMAAC-Biomass Substrate Induced Respiration (SIR)

We also compared LI-COR 8100 and SMAAC measurements during a test designed to mimic SIR measurements (Fierer et al., 2003; Strickland et al., 2010). Refrigerated soil samples from the fields were brought to room temperature overnight. We placed 80 g (equivalent dry mass) of 4-mm sieved soil samples into a



**FIGURE 2 |** Measurement setups for: **(a)** SMAAC-Biomass substrate induced respiration (SIR) measurement; **(b)** SMAAC-Field flux measurement with SMAAC simultaneously located within the LI-COR 8100 sampling chamber; **(c)** SMAAC-Field flux measurement with SMAAC independent of the LI-COR unit; and **(d)** SMAAC-Burst laboratory CO<sub>2</sub> burst measurement.



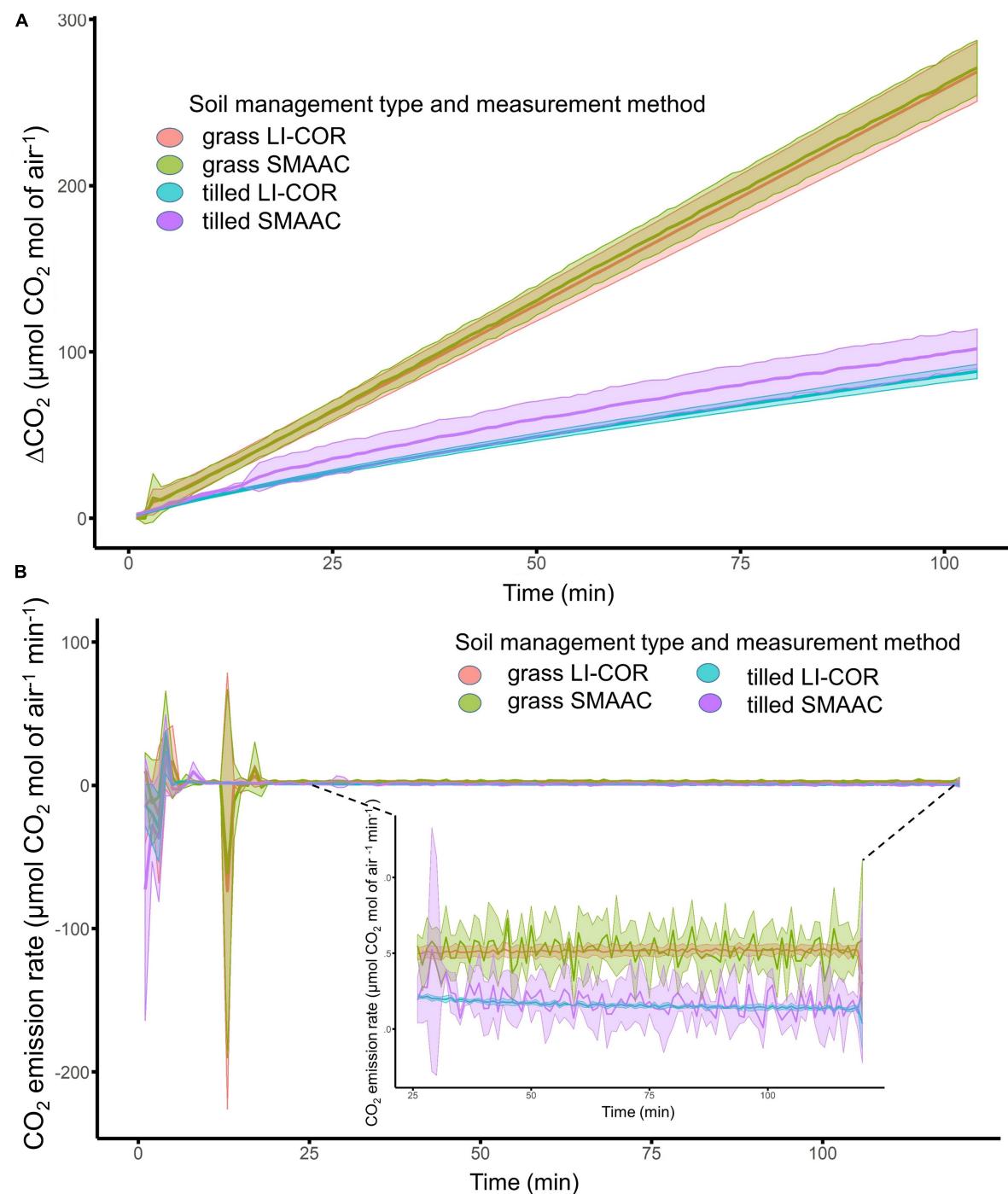
**FIGURE 3 |** CO<sub>2</sub> fluxes measured in the field by the LI-COR (blue), SMAAC-simultaneous (green), and SMAAC-independent (orange). Different small letters indicate grass-covered soil fluxes are statistically different; different capital letters indicate tilled soil fluxes are statistically different (ANOVA with Tukey's HSD;  $P < 0.05$ ).

1 L glass jar (**Figure 2a**). We then added 0.16 L of autolyzed yeast solution made from 12 g of yeast extract (BD Biosciences, San Jose, CA, United States) in 1 L of DI water as a substrate. The mixture of soil and substrate was shaken with no cover for 10 min. We then sealed the jar using a rubber stopper that had the SMAAC sensor and a septum mounted through it. Using the septum, we flushed the headspace of the jar using CO<sub>2</sub> free air for 7 min. Then the jar was maintained at 20°C for 4 h. After 4 h, we collected a gas sample through the septum using a syringe. This sample was injected into the LI-COR 8100 unit to quantify the CO<sub>2</sub> concentration in the jar headspace. The 4-h CO<sub>2</sub> reading from the SMAAC was also analyzed. Both measurements of headspace CO<sub>2</sub> were converted to SIR units ( $\mu\text{g C g}^{-1} \text{ dry soil h}^{-1}$ ) based on the dry mass of soil. Three replicates

were analyzed for the grass buffer and moldboard plowed soils ( $n = 3$ ).

## Statistical Analyses

All statistical analysis and figures were done in R Version 3.5.0 (R Development Core Team., 2018). Analysis of variance (ANOVA) was used to compare the three types of measurements performed in the SMAAC-Field configuration (i.e., LI-COR, SMAAC-simultaneous, and SMAAC-independent). During the SMAAC-Burst and SMAAC-Biomass tests, the Student's *t*-test was used to compare results from the LI-COR vs. the SMAAC. Measurements were analyzed separately for the grass-covered and tilled soils.  $\alpha = 0.05$  was used to test for significance throughout this study.



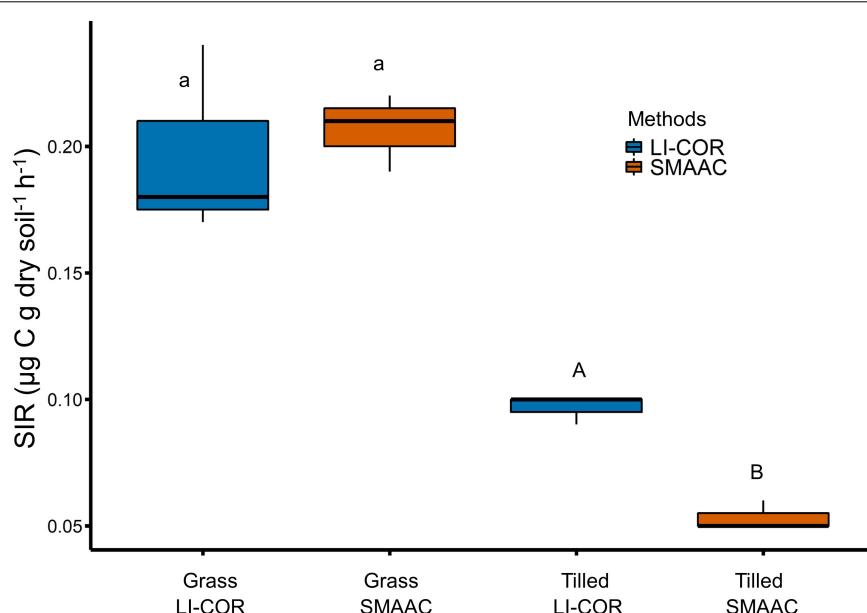
**FIGURE 4 | (A)** Laboratory measurement of change in  $\text{CO}_2$  through time (min) for grass-covered and tilled soils, measured using a LI-COR 8100, and the SMAAC-Burst configuration. Solid lines represent mean values, and shaded areas represent standard deviations from the means. **(B)**  $\text{CO}_2$  emission rates for grass-covered and tilled soils, measured using the LI-COR, and the SMAAC-Burst configuration.

## RESULTS

### SMAAC-Field Soil Respiration Test

For the SMAAC-Field respiration test, the LI-COR 8100 and SMAAC were used to quantify  $\text{CO}_2$  flux over a 2-min period, with

the SMAAC both placed within (SMAAC-simultaneous) and without (SMAAC-independent) the LI-COR sampling chamber. Both instruments showed that the grass-covered soil had a higher  $\text{CO}_2$  flux than the tilled soil (Figure 3). The flux measured for the grass buffer soil by the LI-COR ( $4.1 \times 10^{-4} \mu\text{mol}$



**FIGURE 5 |** Substrate induced respiration measured by a LI-COR 8100 (blue) vs. the SMAAC-Biomass configuration (orange). Different small letters indicate grass-covered soil fluxes are statistically different; different capital letters indicate tilled soil fluxes are statistically different (Students *t*-test;  $P < 0.05$ ).

$\text{CO}_2 \text{ cm}^{-2} \text{ s}^{-1} \pm 9.9 \times 10^{-5}$  standard deviation, SD) was not significantly different than fluxes determined via the SMAAC-simultaneous ( $5.6 \times 10^{-4} \mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1} \pm 2.7 \times 10^{-4}$  SD) or SMAAC-independent ( $3.1 \times 10^{-4} \mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1} \pm 7.2 \times 10^{-5}$  SD) tests. For the tilled soil, the LI-COR flux ( $5.9 \times 10^{-5} \mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1} \pm 1.9 \times 10^{-5}$  SD) was again not significantly different from the fluxes measured during the SMAAC-simultaneous ( $5.9 \times 10^{-5} \mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1} \pm 1.5 \times 10^{-5}$  SD) and SMAAC-independent ( $4.7 \times 10^{-5} \mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1} \pm 2.6 \times 10^{-5}$  SD) tests.

### SMAAC-Burst $\text{CO}_2$ Burst Test

The SMAAC-Burst configuration produced consistent results compared to the LI-COR 8100 unit for both the grass-covered and tilled soils (Figure 4), with similar mean values and standard deviations calculated from the four physical replicates for each soil (Figure 4A). We observed relatively large fluctuations in  $\text{CO}_2$  emission rates, especially during the first 20 min of the experiment (Figure 4B). After this initial period,  $\text{CO}_2$  emission rates fluctuated more for SMAAC compared to LICOR, though the mean rates were generally consistent between methods (Figure 4B). Both instruments showed that the  $\text{CO}_2$  burst was larger in the grass-covered soil compared to the tilled soil (Figure 4).

### SMAAC-Biomass Substrate Induced Respiration Test

Results generated using both the LI-COR 8100 and the SMAAC-Biomass consistently showed that the grass-covered soil had higher SIR values than the tilled soil (Figure 5). The LI-COR ( $0.19 \mu\text{g C g dry soil}^{-1} \text{ h}^{-1} \pm 0.03$  SD) and SMAAC ( $0.21 \mu\text{g C g dry soil}^{-1} \text{ h}^{-1} \pm 0.01$  SD) measurements were not statistically different for the grass-covered soil ( $P \geq 0.05$ ). However, the LI-COR SIR value ( $0.09 \mu\text{g C g dry soil}^{-1} \text{ h}^{-1} \pm 0.005$  SD) for the tilled soil was significantly higher than the SMAAC SIR value ( $0.05 \mu\text{g C g dry soil}^{-1} \text{ h}^{-1} \pm 0.005$  SD;  $P = 0.0009$ ).

$\text{g dry soil}^{-1} \text{ h}^{-1} \pm 0.01$  SD) measurements were not statistically different for the grass-covered soil ( $P \geq 0.05$ ). However, the LI-COR SIR value ( $0.09 \mu\text{g C g dry soil}^{-1} \text{ h}^{-1} \pm 0.005$  SD) for the tilled soil was significantly higher than the SMAAC SIR value ( $0.05 \mu\text{g C g dry soil}^{-1} \text{ h}^{-1} \pm 0.005$  SD;  $P = 0.0009$ ).

## DISCUSSION

In this study we developed three configurations of an Arduino-based  $\text{CO}_2$  sensor that allowed us to assess soil microbial activity. Our instrument, deemed the SMAAC, was then compared against a commercial IRGA unit (LI-COR 8100). Overall, the SMAAC generated similar results to the commercial IRGA, with significant differences only observed when SIR was quantified for the tilled soil (Figure 5). In this example, the SIR value from the SMAAC-Biomass configuration was approximately half of the value estimated by the LI-COR. The reason for the discrepancy may relate to the accuracy of the SMAAC IRGA sensor (50 ppm per the manufacturer). Even though our calibration analysis determined that the instrument provided consistent readings for  $\text{CO}_2$  concentrations between 100 and 500 ppm, the sensor accuracy implies that the error can exceed 10% for  $\text{CO}_2$  concentrations  $< 500$  ppm. Using the sensor to measure low  $\text{CO}_2$  concentrations may therefore require extra precautions such as using longer run times, greater number of replicates, and more frequent calibration. We also note that we did not test the sensor beyond 1,000 ppm, so the calibration should also be assessed when using SMAAC to measure higher  $\text{CO}_2$  concentrations.

The SMAAC tended to show more measurement noise than the LI-COR when assessing  $\text{CO}_2$  fluxes, e.g., the field flux

measurements from SMAAC-simultaneous vs. LI-COR setups in the grass-covered soil (**Figure 3**), or the emissions rates calculated for both soils with the SMAAC-Burst (**Figure 4B**). However, during the field flux measurements, the SMAAC-independent test had a slightly lower median flux and a smaller standard deviation than either the LI-COR or SMAAC-simultaneous. This result may reflect the influence of the LI-COR pump unit, which provided continuous circulation of air in the chamber. At the same time, our flux calculations (Eq. 1) assumed that the volume of the air,  $V_c$ , for the LI-COR and SMAAC-simultaneous setups was equal to the LI-COR sampling chamber plus the internal pump volume of the LI-COR. We did not account for the volume or the exposed surface area of the soil occupied by the SMAAC itself, thus potentially introducing minor error into the flux calculations for those tests. We also note here that the SMAAC and LI-COR both showed high variability in emissions during the initial 20 min of the CO<sub>2</sub> burst test experiment. This result may reflect an equilibration period within the glass jar, particularly in response to the initial soil disturbance during wetting the soil and sealing the system.

The total cost of the SMAAC was ~\$150, making it at least two orders of magnitude less expensive than commercial IRGA units. Despite the low cost, the SMAAC still maintained reasonable accuracy in all three configurations tested, and performed repeatable measurements when compared with CO<sub>2</sub> standards. The SMAAC is lighter weight and requires less power than commercial IRGA units, increasing its usefulness when performing extended measurements or working in remote locations. An additional benefit of the SMAAC comes from its small form factor: it can be placed directly inside the headspace of samples, thus eliminating the need to pull discrete gas samples using a syringe. Removing this step eliminates a potential source of error, particularly since many commercial IRGA pump units are not fully sealed.

The SMAAC may open new avenues of inquiry related to soil respiration measurements, both in terms of the configurations shown here as well as other possible configurations yet to be developed. For example, we focused our tests on closed chamber measurements, since those are commonly used to evaluate soil CO<sub>2</sub> fluxes, and perform measurements such as SIR. The closed chamber measurements also lend themselves to direct comparison with the commercial IRGA unit. However, CO<sub>2</sub> can also be measured using open systems (Norman et al., 1997; Alterio et al., 2006) or in continually flushed chambers (Chow et al., 2006). Using the SMAAC in open/purged systems thus represents an area of possible future development.

Similarly, since the SMAAC system is inexpensive and easy to assemble, multiple sensors could be used concurrently to better quantify spatial, and temporal variability in soil biological measurements, for example by analyzing multiple chambers simultaneously and thereby providing similar functionality as multiplexer units often offered with commercial IRGAs. Finally, direct continuous logging of CO<sub>2</sub> evolution during measurements may help generate new insights. For example, the grass-covered vs. tilled soil showed different temporal trends in the SMAAC-Burst test (**Figure 4B**), where the grass-covered soil produced a constant CO<sub>2</sub> efflux rate over the 2-h test period

vs. a decreasing CO<sub>2</sub> efflux rate for the tilled soil. While the underlying mechanisms controlling these different responses remain beyond the scope of this current paper, it is nonetheless worth noting that it would not be possible to observe such trends without the high measurement frequency offered by IRGA-based instruments such as SMAAC.

## CONCLUSION

The SMAAC developed in this study represents a low cost yet reliable way to measure CO<sub>2</sub> fluxes from soils. The results obtained from the SMAAC were consistent with those from a commercial IRGA unit for both field and laboratory measurements. In this study we highlighted three SMAAC configurations that were designed to assess different aspects of soil microbial activity and function, yet the SMAAC also has the potential to generate additional applications and insights. As an example, by having the SMAAC-Burst and SMAAC-Biomass units placed inside the closed headspace above samples, we generated near-continuous measurements of CO<sub>2</sub> evolution through time. Such CO<sub>2</sub> trends may provide new understanding of soil microbial processes that is not possible via traditional discrete measurements. In conclusion, the SMAAC is a promising tool for measuring soil respiration and microbial activity that warrants usage by the broader scientific community.

## DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

## AUTHOR CONTRIBUTIONS

AJG contributed to designing and conducting the experiments, performing the analyses, and writing the first draft of the manuscript. BL contributed to making the sensor, conducting the experiments, and editing the manuscript. RS contributed to generating the main idea, providing guidance throughout the experiments and analysis, and writing and editing the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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